

Determining Vitreous Subclasses of Hard Red Spring Wheat Using Visible/Near-Infrared Spectroscopy¹

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ABSTRACT

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The percentage of dark hard vitreous (DHV) kernels in hard red spring wheat is an important grading factor that is associated with protein content, kernel hardness, milling properties, and baking quality. The current visual method of determining DHV and non-DHV (NDHV) wheat kernels is time-consuming, tedious, and subject to large errors. The objective of this research was to classify DHV and NDHV wheat kernels, including kernels that were checked, cracked, sprouted, or bleached using visible/near-infrared (Vis/NIR) spectroscopy. Spectra from single DHV and NDHV kernels were collected using a diode-array NIR spectrometer. The dorsal and crease sides of the kernels were viewed. Three wavelength regions, 500–750 nm, 750–1,700 nm, and 500–1,700 nm were compared. Spectra were analyzed by using partial least squares (PLS) regression.

The content of dark hard vitreous (DHV) kernels is a widely used quality factor in grading and marketing of hard red spring wheat (HRS). The DHV kernels usually have a natural translucent color and high hardness index value. The vitreousness of the DHV kernels is related to protein content and hardness (Dexter and Edwards 1998).

In the United States, DHV kernels are considered as subclasses of HRS wheat (USDA 1997). The Grain Inspection, Packers and Stockyard Administration (GIPSA) uses specific criteria to visually determine the subclasses of HRS wheat. Kernels from an HRS sample are considered DHV if they are 1) dark, hard, and vitreous; 2) bleached but still hard and vitreous; 3) checked or cracked with cloudy or shadowy spots but are still dark, hard, and vitreous; or 4) hard red winter and soft red winter wheat kernels within the HRS sample, but still dark, hard, and vitreous. Kernels are considered non-DHV (NDHV) if they are 1) yellow or contain a mottled spot; 2) green immature kernels; 3) severely affected by scab; 4) sprouted; or 5) hard white, soft white, or durum wheat. Foreign material also is classed as NDHV. Three subclasses of HRS wheat are defined by the percentage by weight of DHV kernels present in a 15-g representative sample that is dockage-free with no shrunken or broken kernels. Wheat is assigned to the subclasses of dark northern spring wheat if it contains $\geq 75\%$ DHV kernels; northern spring wheat if it contains 25–75% DHV kernels; or red spring wheat if it has $< 25\%$ DHV kernels. The current visual method of determining DHV and NDHV wheat is time-consuming, tedious, and subject to large errors, and inspectors may not agree on classifications (Dexter and Edwards 1998). Therefore, an objective and reproducible means of determining DHV and NDHV kernels is needed.

Near-infrared (NIR) spectroscopy has been used successfully to measure wheat characteristics such as hardness (Delwiche 1993); protein content (Delwiche 1995, 1998); wheat class (Delwiche and Massie 1996); color class (Wang et al 1999a,b); insect damage (Dowell et al 1998, 1999a); and scab (Dowell et al 1999b). Dowell (2000) studied the possibility of using NIR spectroscopy to identify vitreous and nonvitreous single kernels of durum wheat. Those results showed that NIR spectroscopy could be used to quantify the

Results suggest that the major contributors to classifying DHV and NDHV kernels are light scattering, protein content, kernel hardness, starch content, and kernel color effects on the absorption spectrum. Bleached kernels were the most difficult to classify because of high lightness values. The sample set with bleached kernels yielded lower classification accuracies of 91.1–97.1% compared with 97.5–100% for the sample set without bleached kernels. More than 75% of misclassified kernels were bleached. For sample sets without bleached kernels, the classification models that included the dorsal side gave the highest classification accuracies (99.6–100%) for the testing sample set. Wavelengths in both the Vis/NIR regions or the NIR region alone yielded better classification accuracies than those in the visible region only.

vitreousness of durum wheat, possibly because of protein, starch, or scattering effects on NIR absorption. The objective of this research was to classify HRS DHV and NDHV kernels, including kernels that are checked, cracked, sprouted, and bleached by using visible/near-infrared (Vis/NIR) spectroscopy.

MATERIALS AND METHODS

Wheat Samples

Fourteen wheat samples (100 g) were obtained from GIPSA and separated into DHV kernels and NDHV kernels by a four-member panel from the Board of Appeals and Review (BAR) section of GIPSA (Table I). Each kernel was placed into the DHV or NDHV class by a unanimous decision of the panel. Forty kernels from each sample were randomly selected for Vis/NIR analysis.

Spectra Collection

Spectra from DHV and NDHV single kernels were collected using a diode-array spectrometer (DA7000, Perten Instruments, Springfield, IL). This spectrometer measures extended visible (400–750 nm) and NIR (750–1,700 nm) reflectance at a rate of 30 spectra per second. Single kernels were placed in a black V-shaped trough (12 mm long, 10 mm wide, and 5 mm deep) and illuminated with a quartz tungsten halogen (QTH) light through a fiber bundle (8 mm diameter) positioned 13 mm from the top of the trough and oriented 45° from vertical. The reflectance probe (2 mm diameter) was oriented vertically 9.5 mm from the top of the trough. The reflectance probe carried the reflected energy to a spectrometer. Wheat kernels (560 from 14

TABLE I
Wheat Samples Used for Classification of Dark Hard Vitreous (DHV) and Non-DHV Wheat Kernels^a

DHV Wheat Samples	NDHV Wheat Samples
Clean HRS	Clean HRS
Bleached HRS	Bleached HRS
Cracked HRS	Sprouted HRS
HRW	SRW
Unique HRW ^b	HRW
Cracked HRS	SWH
	HDWH
	Durum

^a $n = 560$ from 14 wheat samples; HRS = hard red spring; HRW = hard red winter; SRW = soft red winter; SWH = soft white; HDWH = hard dark white; and 40 kernels were used for each wheat sample.

^b Unique HRW is a California hard red spring wheat classified as HRW.

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wheat samples) were measured with two orientations: dorsal side and crease side. For each kernel, eight spectra per orientation were collected and averaged. The wavelength regions 500–750, 750–1,700, and 500–1,700 nm were used to differentiate DHV and NDHV wheat kernels. A spectrum of the empty trough, used as a baseline, was measured at the beginning of the test and again after every 100 kernels.

Kernel Color Measurement

Reflectance spectra from 480 to 750 nm were transferred into $L^*a^*b^*$ color space. Grams/32 (Galactic Industries, Salem, NH) software was used for kernel color determination. In the $L^*a^*b^*$ color space, L^* varies from 0 (black) to 100 (perfect white); a^* ranges from -100 to 100 and measures green when negative and red when positive; b^* varies from -100 to 100 and is a measure of blue when negative and yellow when positive.

Statistical Analysis and Model Development

Spectra were analyzed using partial least squares (PLS) regression from the Grams/32 software package. All spectra were mean centered before analysis. PLS is a multivariate data analysis technique designed to handle intercorrelated regressors. Two-class models were developed to classify DHV and NDHV wheat kernels. For a single orientation (crease or dorsal), kernels were separated equally into calibration and testing sets based on even and odd numbers. For two orientations (crease and dorsal), the spectra used from the crease side were from odd-numbered kernels and the spectra used from the dorsal side were from even-numbered kernels in the calibration and testing sample sets, respectively. The DHV kernels and NDHV

kernels were assigned constant values of 1.0 and 2.0, respectively. A kernel was considered to be correctly categorized if the predicted value was below or above 1.5, respectively. Model performance is reported as the cross-validation of each calibration sample set and prediction of test sample sets. The number of PLS factors reported was the minimum required to give the best classification results.

RESULTS AND DISCUSSION

Classification results of cross-validation of calibration sample sets and prediction of testing sample sets with and without bleached kernels using PLS models are summarized in Tables II and III, respectively. To identify the most difficult to classify wheat samples, the percentage of correctly classified individual DHV and NDHV wheat samples corresponding to the testing results in Tables II and III was compared in Tables IV and V. In general, the classification accuracies were lower for the sample set with bleached kernels than for the sample set without bleached kernels. Kernel orientation and wavelength region had a significant effect on the classification accuracy.

Effects of Bleached Kernels on Classification of DHV and NDHV Wheat

With the same PLS factors, the sample set with bleached kernels yielded lower classification accuracies (90.7–97.1%) compared with the sample sets without bleached kernels (97.1–100%) (Tables II and III). The performances of PLS models on the testing sample sets showed similar results. The classification accuracies for the testing sample sets with bleached kernels (90.7–97.1%) were lower

TABLE II
Summary of Cross-Validation and Testing Results Using Partial Least Squares (PLS) for Classification of Dark Hard Vitreous (DHV) and Non-DHV Wheat Kernels Including Bleached Kernels^a

Spectra Region	F^b	Cross-Validation (%)			Testing Results (%)		
		DHV	NDHV	Average	DHV	NDHV	Average
500–750 nm							
Dorsal side	5	97.5	91.3	93.9	95.0	93.1	93.5
Crease side	5	89.2	92.5	91.1	91.6	90.0	90.7
Both sides	8	88.3	92.5	90.7	89.2	92.5	91.1
750–1,700 nm							
Dorsal side	9	96.7	96.5	95.4	96.7	93.8	95.0
Crease side	9	95.8	96.8	96.4	93.3	97.5	95.7
Both sides	12	96.7	97.5	97.1	96.7	97.5	97.1
500–1,700 nm							
Dorsal side	9	96.7	96.5	95.4	95.8	95.0	95.3
Crease side	9	95.8	96.8	94.4	91.7	96.3	94.3
Both sides	15	95.8	95.6	95.7	91.7	96.3	94.3

^a Cross-validation sample set $n = 280$, and testing sample set $n = 280$.

^b Number of PLS regression factors.

TABLE III
Summary of Cross-Validation and Testing Results Using Partial Least Squares (PLS) for Classification of Dark Hard Vitreous (DHV) and Non-DHV Wheat Kernels Excluding Bleached Kernels^a

Spectra Region	F^b	Cross-Validation (%)			Testing Results (%)		
		DHV	NDHV	Average	DHV	NDHV	Average
500–750 nm							
Dorsal side	5	100	100	100.0	100.0	100.0	100.0
Crease side	5	98.0	96.2	97.1	99.0	96.4	97.5
Both sides	8	100	98.6	99.2	99.0	98.6	98.8
750–1,700 nm							
Dorsal side	9	100	100	100.0	100.0	100.0	100.0
Crease side	9	99.0	97.8	98.3	99.0	97.9	98.3
Both sides	12	100	100	100.0	99.0	99.3	99.2
500–1,700 nm							
Dorsal side	9	100	100	100.0	99.0	100.0	99.6
Crease side	9	99.0	97.8	98.3	99.0	98.6	98.5
Both sides	15	100	99.2	99.6	100.0	99.3	99.6

^a Cross-validation sample set $n = 240$, and testing sample set $n = 240$.

^b Number of PLS regression factors.

than those for the testing sample sets without bleached kernels (97.5–100%). More than 75% of misclassified kernels were bleached kernels. This probably was because of the discoloration of the bleached kernels. Bleached kernels had lower *a* and *b* values than unbleached kernels for both DHV and NDHV kernels in the $L^*a^*b^*$ color space (Table VI). This may reduce the spectral differences between DHV and NDHV kernels for bleached kernels. No significant difference in the L^* values was found between NDHV and bleached kernels. This may result in classifying NDHV kernels and bleached kernels into the same class. Taken together, these results indicate that the bleached kernels were the most difficult to classify.

The percentage of correctly classified individual DHV and NDHV wheat samples corresponding to the testing results in Table II is shown in Table IV. Bleached wheat samples had the lowest average classification accuracies (63.3% for bleached DHV kernels and 64.4% for bleached NDHV kernels) among the individual wheat samples. This result offers further indication that the bleached wheat samples are the most difficult to classify for both DHV and NDHV wheat. For DHV wheat samples, unique hard red winter was second most difficult to classify and its classification accuracy (97.8%) was lower than that of unbleached wheat samples. For

NDHV wheat samples, clean hard red spring wheat was second most difficult to classify with a classification accuracy of 95.0%, also lower than that of unbleached wheat samples. Classification accuracies of individual DHV and NDHV wheat samples without bleached wheat corresponding to the testing results in Table III are shown in Table V. For DHV wheat samples, unique hard red winter wheat was the only one difficult to classify, its classification accuracy (96.7%) was lower than other wheat samples. This result is consistent with the results in Table IV. For NDHV wheat samples, clean hard red spring and sprouted hard red spring wheat samples yielded lower classification accuracies (96.1 and 96.7%, respectively) than the other wheat samples.

Effects of Kernel Orientation and Wavelength Region on Classification of DHV and NDHV Wheat Kernels

In general, the average classification accuracies with the dorsal side were better than those for the crease side for both cross-validation of calibration sample sets and prediction of testing sample sets, especially for sample sets without bleached wheat samples (Tables II and III). The classification models including both the dorsal and crease sides gave better classification accuracies than the models with the crease side only. At three wavelength regions,

TABLE IV
Comparison of % Correctly Classified Individual Dark Hard Vitreous (DHV) and Non-DHV Wheat Samples Corresponding to Testing Results in Table II

Wheat Samples*	500–750 nm			750–1,700 nm			500–1,700 nm			Avg. (%)
	Dorsal	Crease	Both	Dorsal	Crease	Both	Dorsal	Crease	Both	
DHV										
Clean HRS	100	100	100	100	100	100	100	100	100	100
Bleached HRS	70.0	50.0	40.0	80.0	75.0	80.0	75.0	50.0	50.0	63.3
Cracked HRS	100	100	100	100	100	100	100	100	100	100
HRW	100	100	100	100	100	100	100	100	100	100
Unique HRW	100	100	95.0	100	85.0	100	100	100	100	97.8
Cracked HRS	100	100	100	100	100	100	100	100	100	100
Avg. (%)	95.0	91.6	89.2	96.7	93.3	96.7	95.8	91.7	91.7	
NDHV										
Clean HRS	95.0	85.0	90.0	100	95.0	100	95.0	95.0	100	95.0
Bleached HRS	50.0	45.0	50.0	50.0	85.0	90.0	65.0	75.0	70.0	64.4
Sprouted HRS	100	95.0	100	100	100	100	100	100	100	99.4
SRW	100	95.0	100	100	100	100	100	100	100	99.4
HRW	100	100	100	100	100	100	100	100	100	100
SWH	100	100	100	100	100	95.0	100	100	100	99.4
HDWH	100	100	100	100	100	95.0	100	100	100	99.4
Durum	100	100	100	100	100	100	100	100	100	100
Avg. (%)	93.1	90.0	92.5	93.8	97.5	97.5	95.0	96.3	96.3	

* HRS = hard red spring; HRW = hard red winter; SWH = soft white; HDWH = hard dark white; *n* = 20 kernels for each wheat sample.

TABLE V
Comparison of % Correctly Classified Individual Dark Hard Vitreous (DHV) and Non-DHV Wheat Samples Corresponding to Testing Results in Table III

Wheat Samples*	500–750 nm			750–1,700 nm			500–1,700 nm			Avg. (%)
	Dorsal	Crease	Both	Dorsal	Crease	Both	Dorsal	Crease	Both	
DHV										
Clean HRS	100	100	100	100	100	100	100	100	100	100
Cracked HRS	100	100	100	100	100	100	100	100	100	100
HRW	100	100	100	100	100	100	100	100	100	100
Unique HRW	100	95.0	95.0	100	95.0	95.0	95.0	95.0	100	96.7
Cracked HRS	100	100	100	100	100	100	100	100	100	100
Avg. (%)	100	99.0	99.0	100	99.0	99.0	99.0	99.0	100	
NDHV										
Clean HRS	100	85.0	95.0	100	95.0	100	100	95.0	95.0	96.1
Sprouted HRS	100	90.0	95.0	100	90.0	100	100	95.0	100	96.7
SRW	100	100	100	100	100	100	100	100	100	100
HRW	100	100	100	100	100	100	100	100	100	100
SWH	100	100	100	100	100	95.0	100	100	100	99.9
HDWH	100	100	100	100	100	100	100	100	100	100
Durum	100	100	100	100	100	100	100	100	100	100
Avg. (%)	100	96.4	98.6	100	97.9	99.3	100	98.6	99.3	

* HRS = hard red spring; HRW = hard red winter; SWH = soft white; HDWH = hard dark white; *n* = 20 kernels for each wheat sample.

the models with the dorsal side only used fewer PLS factors than models with both the dorsal and crease sides together. These results indicate that the dorsal side is the best orientation for classification of DHV and NDHV wheat kernels. This probably was due to the structural differences between the dorsal and crease sides. The depth of the crease varies with different wheat samples and kernel sizes. Some wheat samples have a deep crease, whereas other samples have a flat surface on the crease side. Therefore, it is likely that variations in the depth of crease cause a larger spectral variation than when measured on the dorsal side.

The NIR region (750–1,700 nm) and Vis/NIR region (500–1,700 nm) gave better classification results than the visible region for both cross-validation of calibration sample sets and prediction of testing sample sets (Table VII). Comparisons of correct classification of bleached DHV and NDHV wheat kernels also showed that classification accuracies were higher with wavelength regions of 500–1,700 nm and 750–1,700 nm than with the visible region of 500–750 nm. For bleached kernels, classification accuracies (76.6–80.0%) were significantly higher with the NIR region than with the visible region (50.8–59.9%) and with the Vis/NIR region (64.2–70.0%). This is probably because reflectance spectrum in the visible region represents mostly the surface and physical properties of a measured object, while the reflectance spectrum in the NIR region represents both surface and intrinsic properties of a measured object.

Contributors to Classification of DHV and NDHV Wheat Kernels

Scattering is a major factor contributing to the classification of DHV and NDHV kernels. The average spectra showed that the DHV kernels had higher log (1/R) values than the NDHV kernels (Fig. 1). Dowell (2000) suggested that scattering affects the absorption spectrum because NDHV kernels have air spaces in the endosperm that diffract and diffuse light, which cause them to appear opaque (Hoseney 1986). This results in more incident energy being reflected to the NIR sensor and recorded as lower log (1/R) values for nonvitreous kernels. The DHV kernels are packed more tightly and have fewer or no air spaces to diffract light. Thus, light entering a DHV kernel is more likely to pass through it, resulting in a higher absorbance measured by the NIR sensor. Delwiche (1993) gave a similar explanation for the relationship between vitreousness and optical density.

Figure 2 shows beta coefficients for four-factor PLS calibration models resulting from dorsal side, crease side, and both sides together for the wavelength region of 500–1,700 nm. The beta

coefficients are PLS calibration regression coefficients corresponding to each wavelength used in the calibration model. They indicate which spectral regions are important for prediction and are related to the pure-component spectrum, taking into account all the effects of interfering components, molecular interactions, and baseline variations (Haaland and Thomas 1988). The largest peaks and valleys indicate the most important wavelength regions in the calibration model. The peaks of the beta coefficient curves shifted slightly among different orientations used in the models. However, these curves are mostly in the same pattern, especially for the crease, and crease

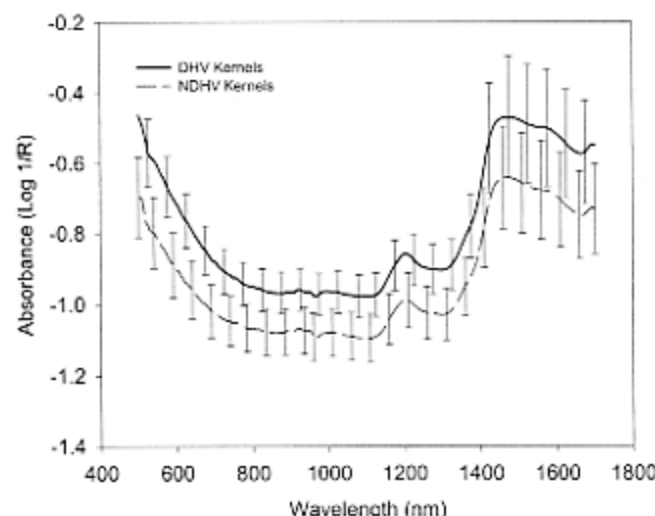


Fig. 1. NIR absorption curves for dark hard vitreous (DHV) and non dark hard vitreous (NDHV) wheat kernels. Each vertical bar represents one standard deviation.

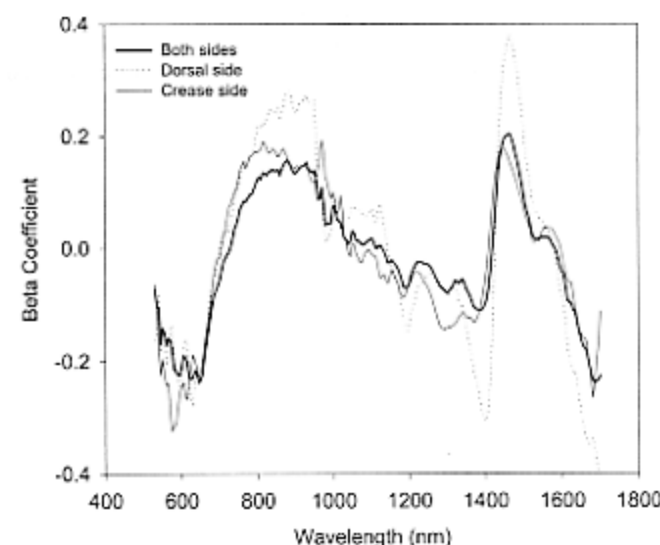


Fig. 2. Beta coefficient curves of partial least squares models (four PLS factors) for classification of dark hard vitreous (DHV) and non dark hard vitreous (NDHV) wheat kernels measured on the dorsal side, crease side, or both.

TABLE VI
Color Variations Between Dark Hard Vitreous (DHV)
and Non-DHV Wheat Kernels
Measured as *L*, *a*, and *b* Values

Class	<i>L</i>	<i>a</i>	<i>b</i>
DHV	43.53a ^a	13.59a	12.07a
NDHV	53.28b	13.01a	11.98a
Bleached DHV	51.56b	11.20b	10.85b
Bleached NDHV	52.13b	10.99b	10.60b

^a Values within the same column followed by different letters are significantly different at *P* < 0.05.

TABLE VII
Effect of Wavelength Regions on Correct Classification
of Dark Hard Vitreous (DHV) and Non-DHV Wheat Kernels

Class	Cross-Validation (%)			Testing Results (%)		
	500–750 nm	750–1,700 nm	500–1,700 nm	500–750 nm	750–1,700 nm	500–1,700 nm
Sample with bleached wheat	91.9	96.3	95.2	91.8	95.9	94.6
Sample without bleached wheat	98.7	99.4	99.0	98.8	99.3	99.2
Bleached wheat sample	59.2	80.0	70.0	50.8	76.7	64.2

and dorsal sides together. Protein content, kernel hardness, starch content, and kernel color are likely the main contributors to the classification of DHV and NDHV kernels. In general, DHV kernels are higher in protein content, lower in starch content, darker in kernel color, and higher in hardness index value than the NDHV kernels (Dexter and Edwards 1998).

The large negative beta coefficients at 550–650 nm were related to wheat kernel color. DHV kernels had a significant lower *L* value than NDHV kernels (Table VI). This indicates that DHV kernels are darker than NDHV kernels. The average *a* value of DHV kernels is higher than that of NDHV kernels. This indicates that some color differences exist between DHV and NDHV wheat samples, which was expected.

The large positive beta coefficients at 770–970 nm were related mostly to protein (Delwiche 1993; Delwiche and Massie 1996; Shenk et al 1992). Large positive beta coefficient values at 1,450–1,540 nm represented absorption bands of moisture, starch, and protein (Shenk et al 1992). The peaks at 1,670 and 1,200 nm occurred around the C-H first and second overtones. The peak at 1,400 nm occurred around the C-H first combination overtone (Murray and Williams 1990). The peaks at =1,330 nm represented kernel hardness (Delwiche and Massie 1996). Additional peaks probably were due to PLS attempts to explain the variation introduced by kernel size, bleaching, checks, and cracks.

In summary, results showed that NIR spectroscopy can be used to classify DHV and NDHV kernels. The major contributors to identifying DHV and NDHV kernel likely are light scattering, protein content, kernel hardness, starch content, and kernel color effects on the absorption spectrum. Bleached kernels were the most difficult to classify. Sample sets with bleached kernels yielded lower classification accuracies than sample sets without bleached kernels. More than 75% of misclassified kernels belonged to bleached wheat samples. Both kernel orientation and wavelength region had significant effects on classification of DHV and NDHV kernels. Classification models based on spectra obtained from the dorsal side gave the best classification accuracies. Classification models with the NIR wavelength region and the Vis/NIR wavelength region yielded better classification results than models with visible wavelength region only.

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